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Pharmacokinetic considerations regarding the treatment of bacterial sexually transmitted infections with azithromycin – A review

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Abstract

Rates of bacterial sexually transmitted infections (STIs) continue to rise, demanding treatments to be highly effective. However, curing infections faces significant challenges due to antimicrobial resistance in *Neisseria gonorrhoeae* and *Mycoplasma genitalium* and especially treating STIs at extragenital sites, particularly rectal chlamydia and oropharyngeal gonorrhoea. As no new antimicrobials are entering the market, clinicians must optimize the currently available treatments, but robust data is lacking on how the properties or pharmacokinetics of antimicrobials can be used to inform STI treatment regimens to improve treatment outcomes. This paper provides a detailed overview of the published pharmacokinetics of antimicrobials used to treat STIs and how factors related to the drug (tissue distribution, protein binding, half-life), human (pH, inflammation, site of infection, drug side effects, sexual practices) or organism (organism load, antimicrobial resistance) can affect treatment outcomes. As azithromycin is commonly used to treat chlamydia, gonorrhoea and *Mycoplasma genitalium* infections and its pharmacokinetics are well studied, it is the main focus of this review. Suggestions are also provided on possible dosing regimens when using extended and/or higher doses of azithromycin, which appropriately balance efficacy and side effects. The paper also emphasizes the limitations of currently published pharmacokinetic studies including oropharyngeal gonococcal infections where very limited data exists around ceftriaxone pharmacokinetics and its use in combination with azithromycin. In future, the different anatomical sites of infections may require alternative therapeutic approaches.

Introduction

Globally, the incidence of the three common bacterial sexually transmitted infections (STIs) chlamydia (*Chlamydia trachomatis,* CT), gonorrhoea (*Neisseria gonorrhoeae;* NG) and syphilis (*Treponema pallidum*) is high and the combined incidence is estimated to be over 215 million new cases among adults (15-49 years) annually.[1] There is also increasing concern about *Mycoplasma genitalium* (MG) infections, which are more common than NG, and in some countries have a similar burden to that of CT infections.[2, 3] These STIs can result in considerable morbidity including reproductive complications in women, increase the risk of HIV transmission, and even cause mortality (e.g. congenital syphilis). [4]

Regular testing enables early detection of infections so that effective treatments can be given to break the onwards transmission and minimise the morbidity associated with infection. However, no single treatment is 100% effective as a result of host factors including patient compliance with taking treatment, vomiting or diarrhoea post-treatment, immune response, and organism factors such as antimicrobial resistance (AMR).

There is increasing concern about antimicrobial treatment failure for several STIs. While bacterial AMR resulting in clinical failure in the treatment of chlamydia is rare or unverified,[5, 6] there is considerable debate questioning the clinical efficacy of 1 gram azithromycin, particularly for rectal CT where repeat infection is common and thought to be frequently due to treatment failure.[7] NG AMR is considered an urgent global threat by the World Health Organization with the organism being resistant to several classes of antimicrobials and dual antimicrobial therapy (mainly ceftriaxone 250-500 mg plus azithromycin 1-2 g) currently recommended as the last remaining option.[8] MG has considerable problems with resistance to both first-line (azithromycin) and second-line (fluoroquinolones, mainly moxifloxacin) treatments.[9 10] In contrast, syphilis continues to be effectively treated with first-line benzathine penicillin G. However, there are increasing

reports of resistance to azithromycin when used in patients with β-lactam allergy and when doxycycline is contraindicated.[11-13]

As concern for global AMR increases and few antimicrobials for STIs are in clinical development,[14, 15] clinicians have little choice but to maximize the efficacy of currently available antimicrobials. One approach is to use higher doses or extended dosing regimens to improve treatment outcomes. However, to improve these dosing regimens an understanding of the pharmacokinetics of therapeutic antimicrobials, including the distribution of the drugs to the anatomical site of infection, is essential.

In this paper, we provide a detailed overview of the published pharmacokinetic characteristics of STI antimicrobials and their impact on treatment efficacy. We highlight gaps in our understanding, with a special emphasis on oropharyngeal infections for which the greatest gaps exist, and discuss how treatment efficacy can be improved by considering the pharmacokinetic properties of antimicrobials. Since azithromycin is frequently used for treating CT,[16] NG,[17] and MG,[9] there is a particular focus on this drug. As first-line treatment (benzathine penicillin G) for syphilis remains highly effective, this STI will not be further addressed.[4]

Key pharmacokinetic parameters of antimicrobials

Pharmacokinetics includes the broad areas of drug absorption, distribution, metabolism and excretion. The primary role of antimicrobial clinical pharmacokinetics is to enhance therapeutic efficacy, decrease drug toxicity, and avoid induction or selection of AMR.

Once a drug is absorbed, either through the oral or parenteral route, it reaches a maximum concentration (Cmax) in the blood/tissues, after a certain time (time to maximum concentration, Tmax). The total amount of drug that is absorbed is defined by the area

under the concentration-time curve over different time intervals: 0-24 hours (AUC_{0-24}), and 0-last time (AUC_{0-last}) and/or 0-infinity ($AUC_{0-\infty}$), the latter corresponding to the estimated total exposure. At the simplistic level, antimicrobial efficacy is measured using pharmacokinetic/pharmacodynamic parameters such as the time the tissue/blood concentration of free drug exceeds the MIC of the microorganism (*f*T>MIC), the ratio of the maximum concentration to MIC (Cmax/MIC), and the ratio of the AUC to MIC (AUC/MIC).[18] Activity of antimicrobials is predominantly time-dependent (*f*T>MIC) for β-lactam antimicrobials (e.g. ceftriaxone) and concentration-dependent (Cmax/MIC and AUC_{0-24}/MIC) for macrolides (e.g. azithromycin), tetracyclines (e.g. doxycycline)[18] and fluoroquinolones (e.g. ciprofloxacin).

Other pharmacokinetic characteristics of importance include bioavailablity, the volume of distribution (Vd) and the half-life ($T_{1/2}$) of a drug. Bioavailability measures the fraction of an administered dose that reaches the systemic circulation. The Vd is the ratio of the dose present in the body and its plasma concentration, when the distribution of the drug between the tissues and the plasma is at equilibrium. In the case of an orally administered drug, this is influenced by the bioavailability of the drug. The larger the Vd, the greater the drug is distributed in the body. Azithromycin and doxycycline both have a very high Vd (>30 L/kg), whereas the Vd is low for ceftriaxone (0.19 L/kg). The $T_{1/2}$ is the time required for the concentration of drug to be reduced by 50% in the blood or tissue. $T_{1/2}$ is a composite measure influenced by the Vd and clearance of the drug. It takes approximately 4-5 half-lives for a drug to reach 'steady state' in the body with conventional dosing regimens i.e. when the rate of drug absorption and elimination are equal. Giving a higher first dose (a 'loading' dose) helps to reach steady state and therapeutic levels faster.

The key pharmacokinetic parameters for antimicrobials commonly used for treatment of STIs are summarized in Table 1.

Table 1. Comparative pharmacokinetics of antimicrobials commonly used for treatment of sexually transmitted infections.

Antimicrobial	**Activity**	**Bioavailability (%)**	**Tmax (hrs)**	**Serum half-life ($T_{1/2}$; hrs)**	**Volume of distribution (Vd; L/kg)**	**Protein binding (%)**	**Predominant excretion**
Azithromycin[19, 20]	Bacteriostatic	Oral: 37	2-3	68	31.1	Concentration dependent: 51% at 0.02 µg/mL to 7% at 2 µg/mL	Bile/faeces
Ceftriaxone[21]	Bactericidal	IM: 100	2-3	6-8 IM: 8.2[22]	0.19[23]	83-96	Bile/faeces (44% of dose)
Doxycycline[21]	Bacteriostatic	Oral: ~100	2-3	12-16	50	82-93	Urine (30-65% dose)
Ciprofloxacin[21]	Bactericidal	Oral: 60-70	1-2	5	3.2	20-40	Urine (40-50%)
Cefixime[21, 24]	Bactericidal	Oral: 40-50	2-6	3-4	1.1	70	Urine (50%)

IM: after intramuscular dose;

Factors affecting the pharmacokinetics and treatment efficacy of antimicrobials

Drug solubility, molecular weight and protein binding

In general, a drug that is more lipid soluble (in contrast to water soluble) such as azithromycin and doxycycline, penetrates across cellular membranes more readily which results in higher tissue penetration and therefore higher Vd. Drugs with low lipid solubility (e.g. gentamicin) will stay mainly in the blood compartment and have lower tissue penetration and Vd. Additionally, drugs of small sizes (low molecular weight) can cross cellular membranes more easily than higher molecular weight drugs.

Drugs that are not bound to protein (i.e. free drug) are pharmacologically active and able to penetrate cells.[25, 26] Protein binding for azithromycin is particularly interesting as unlike most other antimicrobials, protein binding among healthy individuals is dose-dependent, decreasing from 51% at 0.02 μg/mL to 7% at 2 μg/mL,[19] which suggests at high concentrations, protein binding will become saturated resulting in more free drug.

pH effects

Generally, unionised drugs are more lipid soluble and ionised drugs are more water soluble. The degree of ionisation (pKa) affects how much drug is absorbed across cellular membranes and pKa is affected by the pH of the tissue in which the drug is distributed. For example, azithromycin has a pKa of ~8.5[19] meaning at a pH of 8.5, 50% of the drug is ionised and 50% is unionised. Only the unionised form can permeate across cellular membranes and enter a cell,[27] contributing to intracellular concentrations. A one unit increase in the pH from the pKa results in 91% of the drug being unionised, while a one unit decrease results in only 9% being unionised.[28] The optimal effects of macrolides have been suggested to be at a

pH of 8 with a significant decrease in its efficacy at pH values <6.[29] Therefore higher pH levels may be associated with greater tissue penetration leading to greater efficacy than lower pH levels. The pH of the human rectum has been shown to decrease (pH 8 to 7) as a result of inflammatory disease,[30] but it is unknown whether inflammation from an STI infection can result in a similar decrease in pH thereby impacting on treatment efficacy. The implications for the vaginal site are much more complex. A vaginal pH of less than 4.5 is considered healthy[31] and may inhibit chlamydia infection, although how this influences infection at the columnar epithelial surface is unknown.[32] Bacterial vaginosis, which involves a profound shift in the vaginal microbiota to a dysbiotic state, is associated with a higher pH and an increased risk of acquiring other STIs.[33] Inflammation associated with infection will also raise the vaginal pH. It is therefore plausible that higher pH in disease states improves the activity of azithromycin, which is less effective at lower pHs. Efficacy may also be related to the higher pH of the vaginal mucus (median pH of 7, range 5-8)[34] and affected by factors such as menses and sex.[35] It has previously been reported that CT treatment efficacy may be related to the concentration of azithromycin in the cervical mucus.[36]

In the author's study of the pharmacokinetics of azithromycin in rectal tissue,[37] the highest tissue concentrations were observed in the participant who was on long term esomeprazole – a drug that raises intra-gastric pH by reducing acid production. This raises the possibility of role of local pH on the pharmacokinetics of azithromycin.

Effects of inflammation

STIs generate an immune response in the local tissue which may impact on the transport of drugs throughout the body. For example, azithromycin is carried to the site of infection by human phagocytic cells; higher concentrations of azithromycin are reported in diseased

(inflamed) gingiva compared with healthy gingiva (11.6 versus 6.3 µg/g; $p<0.05$)[38] and in inflamed blisters compared with non-inflamed blisters (7.5 versus 4.5 mg.L/hr; $p<0.02$).[39] This suggests that using a higher first dose of azithromycin (*'front end loading'*) with an acute infection when the inflammatory response is most pronounced and phagocytic cells are likely to be most abundant, may result in increased absorption and delivery to an infection site within the first 24 hours causing greater bacterial clearance.[40] This property of azithromycin should be further exploited for STIs.

Site of infection

Evidence shows that drug efficacy can vary by site of infection. For example, azithromycin efficacy for CT appears to be lower in the anorectal site compared with the urogenital site[7, 41] and for NG, treatment efficacy is lowest for oropharyngeal infection. As outlined above, the pharmacokinetics and concentration of a drug can vary by the site of infection as a result of differences in pH of the tissue at the site of infection, protein binding or molecular weight. For example, only free drug or low molecular weight drugs can distribute into saliva which may or may not have an impact on treatment efficacy for oropharyngeal STIs.[42] There is also evidence that the MIC of a drug can vary by infection site with a recent study finding that the CT MIC for azithromycin was about four times higher in a colorectal compared to an endocervical cell line, regardless of CT genovar.[43] For STIs, it is essential that the antimicrobial used effectively distributes to all possible infection sites including oropharyngeal, anorectal or urogenital sites.

Side effects

Drug absorption from the oral route is more sensitive to external factors than drugs given parenterally. For a drug that has low oral bioavailability such as azithromycin (37%), factors that lower its absorption such as vomiting and diarrhoea remain critical for treatment efficacy. Studies show that for a 1 g dose of azithromycin, approximately 2% of subjects experience vomiting when given as a single dose but this is halved when the dose is divided over 3-5 days.[19] NG treatment trials using azithromycin 2 g as a single dose as monotherapy[44] or as part of dual therapies[45] reported vomiting in 6-7% of people. In the author's study of the rectal pharmacokinetics of azithromycin in men, the study found that men who experienced drug side effects such as diarrhoea had lower tissue concentrations,[37] which could contribute to treatment failure.

Organism load

Higher organism load has been associated with treatment failure for several STIs. For example, repeat CT infection following treatment with 1 g azithromycin suggestive of treatment failure has been associated with high organism load infections of the eye,[46,47] vagina,[48] rectum,[49] and pharynx.[50] Another study examining the effectiveness of ciprofloxacin and doxycycline for treating chlamydia urethritis reported significantly higher ciprofloxacin treatment failure rate with infections of higher CT load, but no difference associated with doxycycline.[51] There was no decreased ciprofloxacin susceptibility of the isolates before and after treatment and all patients with ciprofloxacin treatment failure were subsequently cured with seven days doxycycline (100mg twice per day) treatment for one week. For MG infections, higher pre-treatment organism loads have been reported among patients failing treatment with azithromycin,[52] josamycin,[53] and pristinamycin.[54] Further studies have used pre-treatment with doxycycline to reduce the MG load prior to treatment with azithromycin and observed greater cure rates.[55, 56]

Sexual practices

Pre-sex rectal douching with non-isotonic fluids such as water is a common practice among MSM[57] and the use hyperosmolar water-based lubricants have also been shown to cause damage to rectal tissue. Since azithromycin is likely to be found in rectal mucus and faeces,[37] it is plausible that douching may have a reduce treatment efficacy for rectal STIs because douching may reduce local tissue exposure to drug laden faeces or mucus or may remove rectal cells containing azithromycin. [58-60] The author's have previously shown that lower azithromycin rectal concentrations were found in men who practiced rectal douching.[37] Observational studies also suggest that douching is associated with an increased risk of STIs.[57, 61, 62]

Limitations of currently published pharmacokinetic studies

Undertaking and interpreting the results of antimicrobial pharmacokinetic studies is challenging for the following reasons:

i) Tissue concentrations do not always translate into clinical efficacy because of the drug's relative distribution between different tissue compartments, including intracellular and extracellular compartments.[63] This is further complicated because many studies have analysed tissue homogenates only, rather than determining the concentrations within the specific compartments where microorganisms reside, such as in the intracellular space for CT,[64] NG,[65, 66] and MG.[67] Tissue sampling for pharmacokinetic studies is also prone to contamination (e.g. with blood, mucus, other cells) that can lead to overestimation of drug concentrations. In the author's pharmacokinetics studies of 1 g azithromycin in rectal[37] and endocervical tissue,[68] the sampling methods were unable to differentiate both between

intracellular and extracellular space and although the studies were able to report high concentrations of azithromycin, they were unable to report whether it was in the appropriate compartments for chlamydia infection. Further, the studies were unable to differentiate between azithromycin in mucus, blood or cervical/rectal epithelial cellular tissue. Being unable to clearly understanding the compartment in which the organism of interest resides, makes interpretation of results challenging.

ii) MIC is an essential pharmacokinetic/pharmacodynamic parameter, but several factors impact on how accurately it is measured. Firstly, variations in assay methods and bacterial strains can occur. [69] Secondly, as often observed with NG, MICs can change dramatically over time.[70] Thirdly, MICs can vary by tissue type as highlighted when comparing azithromycin MIC for CT grown in colorectal versus endocervical cell lines.[43]

iii) Many studies report total drug concentration only which does not differentiate between protein-bound and free drug.[71] Protein binding may be especially important for oropharyngeal infections because only free drug can distribute into saliva.[72]

Distribution of drug into versus extracellular compartments and its impact on sub-inhibitory antimicrobial levels

Azithromycin

Given it takes about five half-lives for drug concentrations to fall to negligible levels after treatment, patients treated with azithromycin will have a prolonged tail of sub-inhibitory concentrations (sub-MIC) both intra and extracellularly because of the drug's long $T_{1/2}$ (68 hours). This means that the drug will be present for at least 2-4 weeks post treatment at decreasing concentrations over time.[73]

One reason for azithromycin's long intracellular half-life is that after it enters the acidic intracellular compartments the drug is trapped within the cell (ion trapping).[26] While ion trapping helps to concentrate the drug intracellular at the site of infection, it does prevent the drug from diffusing back into the plasma. As a result, concerns remain about the presence of low concentrations in extracellular compartments where organisms may replicate,[74] and where prolonged exposures to sub-inhibitory concentrations could induce or select azithromycin resistance.[75]

We do not know what intracellular drug levels exert induction or selection pressure for resistance in different bacterial species. [26, 73, 76] As the MIC of azithromycin for MG is lower (0.002-0.008 mg/L) than that for NG (0.5 mg/L), it is likely that such an effect would be present for longer *in-vivo* with MG compared with NG. [77, 78] MG develops resistance very easily when using azithromycin monotherapy because it requires mutation of only one gene allele encoding the azithromycin target 23S rRNA. In contrast, NG requires a mutation in four alleles of the 23S rRNA gene.

Dual therapy with an intracellular drug such as azithromycin together with an extracellular drug such as ceftriaxone is useful to target bacteria in both spaces. It has been reported that the $AUC_{0\text{-}24}/MIC_{90}$ for free azithromycin in extracellular compartment was sub-inhibitory for CT following a 500 mg dose.[26] Given that sub-inhibitory concentrations might promote resistance emergence, it would be important to maximize bacterial kill as early as possible during treatment to clear all pathogens throughout the body (intracellularly and extracellularly) by using high loading doses and/or use dual therapy with antimicrobials that together provide high both intracellular and extracellular concentrations, e.g. as in the

recommended dual therapy for gonorrhoea where ceftriaxone plus azithromycin are administered. The choice of loading dose should take the risk of vomiting and diarrhoea and the risk of reduced treatment efficacy into consideration.

Dual therapy for NG using ceftriaxone plus azithromycin is widely recommended.[79] Ceftriaxone resistance is rare, particularly in azithromycin-resistant strains, so any developed azithromycin-resistant gonococcal cells are eradicated by the rapid bactericidal activity of ceftriaxone.[79] With the exception of the UK, azithromycin resistance for NG has remained relatively low internationally[80] and in several countries azithromycin resistance was already present or at higher levels prior to introduction of dual therapy. For example, in Europe, resistance to azithromycin (MIC>0.5 mg/L) was 13.2% in 2009 and 7.2% in 2010, but since dual treatment with ceftriaxone 500 mg and azithromycin 2 g was introduced in 2012, azithromycin resistance has remained stable at 7 to 8% since 2014. [81] The majority of azithromycin-resistant gonococcal isolates in Europe have an azithromycin MIC close to the resistance breakpoint[10] and the clinical relevance of these low-level resistant azithromycin isolates remains unclear.

A further complication of the long half-life of azithromycin is that repeat infection with NG and MG within 2-4 weeks of initial treatment with azithromycin may expose these STIs to sub-inhibitory concentrations that could theoretically promote induction or selection of macrolide resistance. While this is unlikely in low risk individuals, it is a strong probability in high risk populations such as MSM where repeat infection is common.[82, 83] One large study (4660 isolates) reported no association between recent azithromycin exposure and increased azithromycin MICs in cultured gonococcal isolates,[84] but this study was limited as the authors used STI diagnosis as a proxy for azithromycin treatment without knowing

exactly what treatment was prescribed as their national surveillance system did not capture prescribing information. Further, this study did not include rectal infections. However, data from the European gonococcal AMR surveillance shows that azithromycin resistance in rectal NG samples has been lower than that from urogenital and pharyngeal samples.[85]

Ceftriaxone

Increasing the ceftriaxone dose from 500 mg to 1 g to treat NG would extend the duration of ceftriaxone efficacy (*f*T>MIC) but may also result in sub-inhibitory levels. Studies have reported that following a 500 mg intramuscular (IM) dose, ceftriaxone was still detectable in urine 18-36 hours after administration,[86, 87] suggesting reasonable tissue levels would still be present after 36 hours. After a 1 g IM dose, ceftriaxone was detected in tissue (nasal mucosa, tonsil and lung) after 24 hours[88] and in plasma after 36 hours.[22] Another study reported a serum half-life of 17.7 hours following a 1 g IM dose[89] which would equate to 88.5 hours (3.7 days) to clear ceftriaxone. Consideration of the half-life of a drug is important for two reasons. Firstly, ceftriaxone and/or azithromycin concentrations need to be above the MIC for sufficient time to kill NG. Secondly, residual concentrations may pose a risk to induce resistance with re-exposure at sub-MIC levels. A recent modelling paper following a 500 mg and a 1 g ceftriaxone dose reported effective concentrations for ceftriaxone susceptible gonococcal strains (MIC≤0.125 mg/L) in a patient for up to 33 hours and 41 hours, respectively.[70] If a person is re-infected while azithromycin from the NG dual therapy remains present in sub-inhibitory concentrations intracellularly, levels would be insufficient to protect against induction or selection of macrolide resistance in NG. In contrast, the risk of inducing or selecting resistance to ceftriaxone is likely to be less, as the duration of sub-MIC levels and the drugs half-life is shorter than those for azithromycin.

Extended doses may improve treatment efficacy

It is likely that higher doses and/or extended dose regimens of antimicrobials will improve treatment efficacy,[90, 91] by increasing *f*T>MIC, Cmax, decreasing the time to Cmax and increasing the overall AUC, thereby increasing the time the tissue concentrations are above the MIC for the organism. For example, higher doses of azithromycin of 1.5 g or more have been reported to be more effective at treating MG,[55, 56] NG,[44, 92] and CT.[93] In designing extended dose regimens, it is important to ensure a balance between maximizing patient compliance with short courses, minimizing side effects by limiting any single dose, and maximizing effectiveness. It has been demonstrated that for any given total dose of azithromycin, administration as a single or a short course (e.g. duration of less than 3 days) provides similar or better outcomes compared to the same total dose given over a longer period. Data also show that higher systemic exposure of azithromycin was obtained when a total dose of 1.5g was given over 3 days instead of 5 days (19.4 versus 15.9 mg.h/L respectively; p=0.06).[94] Additionally, exposure in the first 24 hours ($AUC_{0\text{-}24}$) was approximately three-fold higher following a single 2 g azithromycin dose compared with a single 500 mg dose (9.3 versus 2.9 mg.h/L).[95-97] Animal studies also report that 'front end' azithromycin dosing produced superior rates of bacterial clearance.[40] These data suggest that the higher the first dose (or 'loading' dose), the greater the systemic exposure of azithromycin in the first 24 hours and thus the likelihood that the treatment will be more efficacious.

While it makes sense that maximizing the first dose with a single 2 g azithromycin dose rather than a 1 g azithromycin dose as part of any extended regimen should improve

treatment efficacy, patient tolerance may be compromised with higher gastrointestinal side effects (35%[44] versus 24%[41] for 2 g and 1 g single dose respectively; p<0.01).[56] It is important to consider other potential adverse events and the populations being treated with extended azithromycin regimens with one study finding a small increased risk of as cardiovascular death in older patients with pre-existing cardiovascular disease,[98] although this was based on observational and not randomized controlled trial data. Nevertheless, the risks and benefits of extended azithromycin doses should be considered.

Gaps in our understanding about the pharmacokinetics of antimicrobials for STIs – the example of oropharyngeal STIs

The role of saliva in oropharyngeal STIs

The pharmacokinetics of antimicrobials in the pharyngeal mucosa is complex and it is not clear what relative antimicrobial concentrations are required in tissue and saliva to cure oropharyngeal STIs, or even whether saliva concentrations are needed at all. [42] Further, little is known about the microbiology of STIs in the oropharynx. Take NG as an example. It has been found intracellularly in tissue sections of tonsils, in cellular debri in tonsillar crypts, in tonsillar exudate and saliva but not in gingiva, buccal mucosa or the tongue.[99] NG organism load has been shown to be similar between the pharnyges and saliva and it can be cultured from the saliva.[100] However, there is little published information about where NG grows and replicates in the oropharynx and what tissues/compartments drugs need to target or what concentrations are needed to cure STIs. We know even less about the microbiology of CT or MG in the oropharynx but saliva has also been reported to have an inhibitory effect on CT *in-vitro*.[101] Yet this information is vital to ensure that oropharyngeal STIs are effectively cured because oropharyngeal environment is ideal for the horizontal transfer of AMR

determinants from co-colonising commensal Neisseria species or other micro-organisms.[102, 103]

Generally only free drug is able to effectively distribute into saliva.[72] For bacteria that are found in saliva such as for NG saliva concentrations impact on treatment efficacy by acting a reservoir of untreated bacteria.[104] Table 2 summarises the level of protein binding and relative concentrations in saliva compared to plasma of antimicrobials used currently or previously for STIs.

Table 2: Relative concentrations of antimicrobials in saliva compared to plasma[38, 42, 88, 90, 105, 106]

Antibiotic	**Protein binding (%)**	**Saliva to plasma ratio***
Azithromycin	7-51**	6
Gentamicin	<30	0.9
Moxifloxacin	50	0.9
Ofloxacin	32	Healthy: 0.8 Sick: 1.4
Amoxicillin	20	0.6
Ciprofloxacin	20-40	0.5
Cefixime	65	0.2
Erythromycin	85	0.2
Doxycycline	82-93	0.1
Ceftriaxone	83-96	<0.004
Penicillin V	80	0 (Not detected)

* Ratio=drug concentration in saliva / drug concentration in plasma; approximate
** Concentration dependent; lower protein binding at higher concentrations

With respect to oropharyngeal NG, the data show that historical treatment with oral ciprofloxacin (having a high cure rate for ciprofloxacin susceptible pharyngeal NG), cefixime and amoxicillin (with probenecid) resulted in low saliva-plasma ratios of 0.6 or less. For injectable ceftriaxone, saliva-plasma ratios were even lower (<0.1) and ceftriaxone has been reported as undetectable in saliva[22] but with concentrations in tonsils (tonsil-plasma ratio 0.2).[88] Accordingly, the high efficacy of ciprofloxacin and ceftriaxone to treat pharyngeal NG is likely related to high distribution into other tissue compartments and/or associated additionally with their rapid bactericidal activity. However, azithromycin reached high levels in saliva/gingiva[38] (gingiva-plasma ratio of 17 and 31 for healthy and inflamed tissue, respectively) and tonsillar tissue (tonsil-plasma ratio of 150),[107] suggesting high oral tissue penetration. This indicates that azithromycin may be highly effective for the treatment of oropharyngeal NG infections and possibly more effective than many other antimicrobials. However, one recent randomised controlled clinical trial (RCT) examining the use of injectable gentamicin 240 mg plus azithromycin 1 g for treatment of NG showed 94% cure rate for urogenital sites but only 90% for anogenital infections and 80% for pharyngeal infections.[108] This may be related to the low distribution of gentamicin in saliva, but also that 1 g of azithromycin may be too low dose in dual NG therapy. In contrast, a RCT evaluating gentamicin 240 mg plus azithromycin 2 g reported 100% cure rate for urogenital infections (n=202) and few pharyngeal (n=10) and rectal (n=1) gonococcal infections although sample size for these last two sites was very small.[45] This combination may have improved efficacy as azithromycin was used in a 2 g dose, and azithromycin is concentrated intracellularly and gentamicin extracellularly.[109] RCTs including larger number of extragenital, particularly pharyngeal, infections and using higher dose azithromycin regimen, perhaps in divided doses, without significantly increasing the prevalence of side

effects (especially vomiting[56]) in combination with ceftriaxone, gentamicin and possibly other antimicrobials would be valuable.

Cellular and saliva turnover in mouth

Drug pharmacokinetics in the mouth and oropharynx requires that we understand the interaction between bacteria, different oral and pharyngeal epithelial surfaces and saliva. A recent human study[110] reported that bacteria can only survive in the mouth if they are attached to epithelial surfaces, that this bacteria-tissue binding is strong, and that most bacteria in saliva is attached to epithelial cells. There are no data available about this for oropharyngeal STIs, but it is plausible that for NG, ceftriaxone works via high concentrations in epithelial cells in the absence of detectable concentrations in saliva. Saliva is constantly being produced and epithelial cells lining the mouth are replaced every 2.7 hours, faster that the rate of bacterial growth. These data suggest two things. Firstly, drugs distributed in saliva will be swallowed (every minute) meaning there is short contact times between bacteria and drugs in saliva. Dosing at night when patients are asleep when there is lower saliva flow could potentially improve efficacy. Secondly, tissue or cell surface mucus concentrations[111] are likely to be more important than saliva since bacteria, even those in saliva are constantly in contact with tissue. Furthermore, the longer the $T_{1/2}$ of a drug, the longer it will be found in tissue and saliva and be less affected by the rapid cycling of epithelial tissue and saliva. Based on this, Tables 1 and 2 show that the pharmacokinetic properties (Vd, saliva-plasma ratio and $T_{1/2}$) for azithromycin is greater than for most other antimicrobials used for treatment of STIs, which supports its antimicrobial value, particularly taking different dosing regimens into consideration, until better options are available

Discussion

Different anatomical sites of infection may require different antimicrobials and/or dosing regimens for the same STI. For CT, current studies show that rectal infections derive substantially greater cure rates using doxycycline[7] rather than azithromycin, although there are currently no RCT data to confirm this. Doxycycline also appears more effective than azithromycin for urogenital CT, although the differences in cure are much smaller. [41] As discussed above, there are many factors contributing to differences in antimicrobial efficacy by tissue site. This may be because antimicrobial concentrations vary by tissues, as seen with higher MIC values for CT in colorectal cells compared to in endocervical cells.[43] It is possible that for pharyngeal NG, higher azithromycin doses may have higher efficacy.[45] Furthermore, for NG, dual therapy (ceftriaxone plus azithromycin) currently appears to provide high efficacy for all sites of infection[17, 79, 112] and it appears effective at minimizing the spread of gonococcal AMR.[79] However, the ideal doses or dosing regimens of ceftriaxone and azithromycin have not been established and other effective antimicrobial combinations might be possible. Hypothetically, high dose azithromycin (2 g) may be advantageous in combination with gentamicin 240 mg[45] as these drugs target the intracellular and extracellular bacteria, respectively, thereby killing any intracellular organisms escaping to the extracellular space, minimizing the impact of sub-inhibitory levels of antimicriobials.[66] A recent study also found that NG can evade the host innate immune response when co-infected with CT[113] so the use of azithromycin with its high intracellular concentrations may benefit also dual infections.

Given the lack of new drugs for STIs entering the market, optimization of current antimicrobials in relation to the dose, dosing regimen, and/or its use as part of combination therapies remains the only feasible option. For intracellular infections such as CT, MG and

NG, azithromycin still remains a useful option but higher doses (at least 2 g), possibly divided over a number of days, are likely required. Higher doses of at least 2 g total are probably resulting in higher concentrations for prolonged duration in all tissue sites and explain better cure rates for NG,[45, 108] MG [56] and likely CT.[114] These regimens should ideally be administered with front end loading doses (i.e. 1 g), which result in higher AUC and greater bacterial clearance and be administered over no more than 3 days (1 g, 500 mg, 500 mg).[40] Dividing the doses also reduces the risk of vomiting which can affect efficacy.

However, higher doses result in a longer duration that an antimicrobial remains in the body after ending treatment and increase the duration of sub-inhibitory concentrations that could increase the risk for inducing or selecting resistance if index cases are re-exposed to bacteria when these are present. This is particularly relevant with azithromycin whose long $T_{1/2}$ means that azithromycin after a 1.5 g dose divided on three consecutive days has been extrapolated, from detection in plasma in up to 14 days after administration, to be present in some body sites for up to 4 weeks.[73] This problem has also been reported for *Streptococcus pneumoniae* exposed to sub-inhibitory azithromycin levels.[115] For STIs, this is mainly a problem for risk groups who are at high risk of re-exposure to STIs within days to weeks following treatment. This risk of resistance emergence may be possible to mitigate by advising no, particularly unprotected, sexual intercourse for at least 2 weeks after completing therapy. However, in high risk groups the compliance to this advice may be limited, and the rates of condom use, especially in oral sex, are suboptimal in both heterosexual and MSM populations.

The influence of pH remains a new area to explore as studies have shown disease (possibly inflammation) can alter the pH in both the mouth (saliva)[116] and rectum[30] of human and the efficacy of azithromycin is pH dependent. Rectal pH may also be changed by

acid lowering drugs such as proton pump inhibitors.[37] Finally, the effects of biofilm formation and clumping of bacteria on treatments of infections in different anatomical sites with STI antimicrobials are mainly unexplored.

In conclusion, an enhanced understanding of the pharmacokinetics of current and future STI therapeutic antimicrobials is essential to guide appropriate STI treatment, particularly when there are few new antimicrobials in development. Gaining detailed insights into the distribution and activity of drugs at the different anatomical sites of infection (oropharyngeal, urogenital and/or rectal) may show that site targeted therapies may be one new method in optimizing current treatment for emerging AMR infections.

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